Cytoplasmic Male Sterility in Two Wild *Helianthus annuus* L. Accessions and Their Fertility Restoration

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ABSTRACT

Commercial sunflower hybrids have been produced by means of a single male-sterile *Helianthus petiolaris* Nutt. cytoplasm and a few fertility restoration genes. The objectives of this study were to characterize cytoplasmic male-sterility (cms) systems in wild *H. annuus* L. accessions (PI 413178 and PI 413180) and to determine the inheritance of fertility restoration. Male-sterile plants were identified and maintained by backcrossing with inbred line HA89. Male-fertile progenies from crosses between cms plants of the two PIs and USDA inbred lines indicated the presence of fertility restoration genes in P21, RMAX1, and PI 413178 for cms PI 413178 (cms-ANN2), and P21, RHA280, RHA801, RPET2, and PI 413180 for cms PI 413180 (cms-ANN3). All heterozygous male-fertile plants of backcross progenies, except for RHA280, crossed to cms plants resulted in a segregation ratio of one male-fertile to one male sterile, indicating a single dominant gene controlling fertility restoration. The backcross progeny of cms PI 413180/HA89//cms PI 413180/RHA280 had a segregation ratio of one male-fertile to three male sterile, suggesting two complementary dominant genes for fertility restoration. Pollinating male-fertile plants of both accessions with HA89 pollen resulted in male-fertile and male-sterile F_1 plants, suggesting the existence of male-sterile cytoplasm and heterozygosity for restoration genes in the male-fertile plants. In field tests, male-sterile PI 413178/4*HA89 and PI 413180/4*HA89 plants produced no seed after self-pollination, and 95 and 98% seed set, respectively, under open-pollination indicating complete male-sterility and female fertility. The new cms sources from wild *H. annuus* and corresponding fertility restoration genes provide diversity for sunflower hybrid production.

A MALE-STERILE CYTOPLASM derived from *Helianthus petiolaris* subsp. *petiolaris* (PET1) and the subsequent identification of dominant fertility restoration genes led to the development and commercialization of sunflower hybrids (Leclercq, 1969; Enns et al., 1970; Kinman, 1970; Vranceanu and Stoenescu, 1970). This source of cms and a few fertility restoration genes, including the widely used Rf_1 and Rf_2 genes, have been used almost exclusively for sunflower hybrid production worldwide (Miller and Fick, 1997). New cms sources and fertility restoration genes are needed to increase the genetic diversity of the commercial hybrids.

Over 60 additional sources of cms have been identified from progenies of crosses between wild *Helianthus* species and cultivated lines (Leclercq, 1983; Whelan and Dedio, 1980; Anashchenko et al., 1974; Serieys, 1991, 1994; Heiser, 1982; Vranceanu et al., 1986; Vulpe, 1972; Christov, 1994), from wild species (Heiser, 1982; Serieys, 1984; Skoric et al., 1987; Jan, 1990; Jan and Zhang, 1994), or from induced mutation (Jan and Rutger, 1988). Fertility restoration genes have been identified in 30 sources, with detailed inheritance studies completed for 17 (Serieys, 1999).

Male-sterile plants were observed in two *H. annuus* accessions in a disease infected nursery near Moorhead, MN. Preliminary studies of these two male-sterile sources and the corresponding fertility restoration genes suggested that these accessions were potentially useful cms sources (Jan, 1990). The objectives of this study were to characterize the cms sources, and to determine the inheritance of pollen fertility restoration.

MATERIALS AND METHODS

A wild *Helianthus* nursery was established near Moorhead, MN, for Sclerotinia wilt [*Sclerotinia sclerotiorum* (Lib.) De Barry] resistance evaluations in the summer of 1987. The 128 entries tested included 124 accessions of wild *H. annuus*, two of *H. petiolaris*, and one each of *H. nuttallii* T. & G. and *H. pauciflorus* Nutt. (=*rigidus*). About 60 plants per entry were established. Typical male-sterile plants, without extruding anthers or pollen production, were observed in two accessions of *H. annuus*, PI 413178 and PI 413180.

Twelve USDA inbred lines and bulk pollen from male-fertile plants of each of the two *H. annuus* accessions were used to pollinate the male-sterile plants of PI 413178 and PI 413180. Additional crosses between wild male-sterile plants or male-sterile F_1 plants and HA89 as the recurrent pollen parent were made in the greenhouse. The F_1 and BC_1F_1 plants grown in the field were scored visually for male fertility. The male-fertile plants had normal anther dehiscence and pollen production and the male-sterile plants showed neither anther extrusion nor pollen production.

Since self-incompatibility in male-fertile F_1 segregants prevented the production of F_2 populations, the male-fertile F_1s were used as the pollen parent in testcrosses to male-sterile plants. The sterile progenies were maintained by backcrossing cms plants with the line HA89. Testcross progenies were scored visually for segregation of male-fertile and male-sterile plants in the field in 1989 and in the greenhouse in 1990 and 1991. The testcross segregation ratios were compared with the theoretical ratio for a 1-gene model by Chi-square analyses. All male-fertile testcross progenies in the 1991 greenhouse planting were evaluated for pollen stainability (Alexander, 1969). Male-sterile plants from the crosses PI 413178/4*HA89 and PI 413180/4*HA89 were grown in the field in 1990. Randomly selected heads were either self-pollinated or open-pollinated. Seed set provided estimates of male and female fertility. The seed set was considered the number of filled seeds divided by the number of florets in each head, expressed as a percentage.

Original seed of PI 413178 and PI 413180 were grown in the greenhouse in 1989 to estimate the frequency of male-sterile plants. Eleven and eight male-fertile plants of PI 413178 and PI 413180, respectively, were emasculated and pollinated with pollen from the line HA89. Fifteen progeny plants for each of the 19 F_1 families were grown in the field in 1991 to examine the possible existence of male-fertile cytoplasms in those 19 male-fertile plants.

Working with lines derived from the same two *H. annuus*

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Table 1. Frequency of F_1 male-fertile (MF) and male-sterile (MS) *H. annuus* plants after crossing MS plants of PI 413178 (cms-ANN2) and PI 413180 (cms-ANN3) with 12 sunflower restoration testers, and male-fertile plants of PI 413178 and PI 413180.

Restoration tester (♂)	F_1 fertility restoration of testers crossed with			
	cms-ANN2†		cms-ANN3†	
	MF	MS	MF	MS
	no.			
HA89	0	50	0	102
P21	3	52	11	109
RHA266	0	34	0	72
RHA274	0	35	0	137
RHA280	0	29	10	70
RHA282	0	57	0	58
RHA294	0	62	0	57
RHA296	0	66	0	74
RHA801	0	58	3	60
RPET2	0	33	1	63
RGIG1	0	38	0	129
RMAX1	1	23	0	159
MF PI 413178	7	9	–	–
MF PI 413180	–	–	25	56

† The cms-ANN2 parent included MS plants of PI 413178, PI 413178/HA89, and PI 413178/2*HA89. The cms-ANN3 parent included MS plants of PI 413180, PI 413180/HA89, and PI 413180/2*HA89.

accessions, Serieys (1991, personal communication) pollinated cms plants with 18 fertility restoration testers, including 16 USDA lines and two French lines, and observed only male-sterile plants in the F_1 progenies. He designated the cms cytoplasm derived from PI 413178 as cms-ANN2 and the cms cytoplasm from PI 413180 as cms-ANN3 (Serieys, 1991). The cms sources described in this report were discovered independently and without knowledge of the cms lines identified by Serieys.

RESULTS AND DISCUSSION

Greenhouse grown plants of the PI 413178 and PI 413180 accessions produced a high frequency of male-sterile progenies: 12 male-fertile and six male-sterile plants (PI 413178), and 12 male-fertile and nine male-sterile plants (PI 413180). Complete male sterility was observed in progenies after backcrossing non-restoring recurrent parents to both accessions, indicating the cytoplasmic control of male sterility. A low frequency of dominant nuclear fertility restoration genes was identified in some USDA inbred lines involved in the crosses (Table 1). P21 and RMAX1 contained fertility restoration genes for cms PI 413178, and P21, RHA280, RHA801, and RPET2 provided fertility restoration genes for cms PI 413180. When a fertility restoration gene exists at a low frequency in a line, pollination with bulk pollen of many plants of the respective line onto the cms sources, and large F_1 populations increase the probability of its identification.

The suspected rarity of fertility restoration genes in cultivated lines for cms-ANN2 and cms-ANN3 is evident. Not surprisingly, male-fertile plants of PI 413178 and PI 413180 had the highest frequency of restoration genes from their respective cms sources (Table 1). Seven USDA restoration lines for the cms-PET1 cytoplasm were unable to restore the cms-ANN2 and cms-ANN3 cytoplasms. These two cms sources had different reactions to the restoration testers indicating distinct mechanisms of cytoplasmic male sterility.

A recent study of cms plants in four wild *H. annuus* accessions, PI 406647, PI 413024, PI 413043, and PI 413158, confirmed the high frequency of fertility restoration genes in each accession (Jan, 1990). In another study, plants with reduced vigor occurred in backcross progenies when substituting the nucleus of the line HA89 into the cytoplasms of perennial diploid *Helianthus* species (Jan, 1992). Genes compensating for the cytoplasmic deficiency were recovered from normal segregants and those genes originated from the respective wild species.

Deriving nuclear fertility restoration gene(s) from a wild *Helianthus* species cms donor provides an alternative when restoration genes are not found or are very rare in cultivated genotypes. Backcrossing male-sterile segregants with sterility maintainer lines has as a result total male-sterile progenies. Backcrossing maintainer lines onto male-fertile segregants is a way of developing restoration lines. After sufficient backcrosses, isogenic lines differing only in the presence or absence of restoration genes can be produced by self-pollination of the male-fertile segregants. Isogenic lines plus the maintainer line in the original *H. annuus* cytoplasm will provide material for testing the effects of the new cms cytoplasm as well as the new restoration gene(s).

With the exception of RHA280, in eight cases testcross progenies of male-fertile plants (heterozygous for fertility restoration and with sterile cytoplasms), had segregation ratios of one male-fertile to one male-sterile plant, indicating that a single dominant gene controlled fertility restoration (Table 2). The segregation ratio of

Table 2. Segregation of male-fertile (MF) and male-sterile (MS) *H. annuus* plants, and pollen stainability of testcross progenies of heterozygous MF plants crossed with MS plants in cms cytoplasms of PI 413178 and PI 413180, respectively.

Pedigree†	No. plants		Theoretical segregation ratio			Pollen stainability	
	MF	MS	F:S	χ^2	P	No. of Plants	Mean (Range)
							%
PI 413178/HA89//PI 413178/*P21*	34	35	1:1	0.01	>0.99	9	95(86-99)
PI 413178/HA89 or R274//PI 413178/*PI 413178*	40	55	1:1	2.37	0.10-0.25	8	97(92-99)
PI 413178/4*HA89/3/PI 413178/RPET2//*RMAX1*	23	29	1:1	0.69	0.25-0.50	21	91(78-99)
PI 413180/HA89//PI 413180/*RHA280*	26	63	1:3	0.84	0.25-0.50	–	–
PI 413180/HA89//PI 413180/*PI 413180*	20	19	1:1	0.03	0.97-0.99	–	–
PI 413180/2*HA89//*PI413180*/3/HA89	11	11	1:1	0	>0.99	11	81(53-98)
PI 413180/2*HA89//*P21*/3/HA89	20	30	1:1	2.00	0.10-0.25	13	72(36-95)
PI 413180/4*HA89/3/PI 413180/RPET2//*RPET2*	29	28	1:1	0.02	0.75-0.90	22	81(19-97)
PI 413180/4*HA89/3/PI 413180/2*HA89//*RHA801*	8	12	1:1	0.80	0.25-0.50	–	–

† Parent in italic type in each pedigree provided fertility restoration gene(s).

one male fertile to three male sterile in the testcross involving RHA280 indicated that this line possessed two dominant complementary genes responsible for fertility restoration of the male-sterile PI 413180 cytoplasm.

Low self-compatibility in the progeny makes pollen stainability a better indicator of fertility restoration than the seed set. The heterozygous male-fertile testcross progenies, for fertility restoration genes of P21, PI 413178, and RMAX1, restored pollen stainability to over 90%, indicating complete dominance. For cms PI 413180, restoration genes from PI 413180, P21, and RPET2 produced an average pollen stainability of 81, 72, and 81%, respectively, with a wide range of variation within each testcross combination. These results indicated partial dominance and the presence of modifier genes. Further selection for fertility restoration in these three sources is necessary before being used for commercial hybrid production. Testcross progenies involving RHA801 were not available for pollen evaluation. However, pollen stainability was evaluated in 29 male-fertile plants from a cross between cms PI 413180/3*HA89 and cms PI 413180/2*HA89//RHA801, F_2, and an F_2 plant homozygous for the RHA801 restoration gene. The observed 95% pollen stainability, with a range of 82 to 100%, indicated that the fertility restoration gene in RHA801 conferred nearly complete fertility with dominant gene action.

Since the male-fertile plants of these two accessions also provided fertility restoration genes, 11 and 8 male-fertile plants of PI 413178, and PI 413180, respectively, were pollinated with HA89 to determine if the male-fertile plants also possessed cms cytoplasms. All the F_1 families segregated for male-sterile plants suggesting that all plants in both accessions have male-sterile cytoplasm. Fertility of those male-fertile plants is assumed to be the result of restoration genes. The number of fertility restoration genes in each accession and their allelic relationships have not been determined.

To be useful for hybrid seed production, a cms line needs complete male sterility and female fertility. In our 1990 field planting, cytoplasmic male-sterile plants of PI 413178/4*HA89 produced no seed in 11 heads after self-pollination, and an average of 95% seed set upon open-pollination. Similarly, cytoplasmic male-sterile plants of PI 413180/4*HA89 produced no self-pollinated seed set in 19 heads and an average of 98% seed set in eight open-pollinated heads. These results indicated that both cytoplasm sources had complete male sterility and completely female fertility. Presently, a cms cytoplasm of a related species, *H. petiolaris*, is used for commercial sunflower hybrid production. Adverse cytoplasmic-nuclear interactions are likely to occur between distantly related species. Cytoplasmic-nuclear interactions producing plants with reduced vigor were recorded when the nucleus of the line HA89 was incorporated in the cytoplasm of five diploid perennial *Helianthus* species (Jan, 1992). Since the two new cms sources are from *H. annuus*, the same species as cultivated sunflower, it is supposed it would be less likely to cause adverse cytoplasmic-nuclear interactions affecting commercial production.

This paper describes the isolation of cytoplasmic male sterility system from two wild *H. annuus* accessions, PI 413178 and PI 413180. Single dominant fertility restoration gene(s) were identified for both cms PI 413178 (cms-ANN2), and cms PI 413180 (cms-ANN3). Backcross progeny of cms PI 413178 and cms PI 413180 with HA89 had complete male sterility and female fertility. These represent the first two cms sources from wild *H. annuus* with restoration genes identified and genetically evaluated. They are different from other reported cms cytoplasms and, together with their restoration gene(s), will reduce the genetic vulnerability of sunflower hybrids by providing an alternative to the cms-PET1 cytoplasm.

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A *fap7* Allele for Elevated Palmitate in Soybean

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ABSTRACT

Soybean [*Glycine max* (L.) Merr.] oil with elevated palmitate content may be useful for food and industrial applications requiring oil with high oxidative stability. The mutant line A30 with ≈ 40 g kg^{-1} greater palmitate content than conventional soybean oil was developed by treating seeds of the line A89-144026 with *N*-nitroso-*N*-methyl urea. This study was conducted to determine the genetic control of elevated palmitate content in A30. Crosses were made between A30, its parent, and lines possessing the *fap1*, *fap2-b*, *fap3*, *fap4*, *fap5*, or *fap6* alleles for altered palmitate. The F_1 seeds from reciprocal crosses between A30 and A89-144026 did not exhibit maternal effects or dominance for palmitate content. The phenotypic and genotypic analyses of F_2 seeds and F_3 progeny indicated that A30 had an elevated palmitate allele, designated *fap7*, with additive gene action at a single locus. The *fap7* locus was independent of *fap1*, *fap2-b*, *fap3*, *fap4*, and *fap5*, but closely linked to the *fap6* locus. The new allele can be used in combination with other alleles that control fatty ester composition to obtain unique oils for possible food and industrial applications.

The palmitate content of soybean oil has been altered by the formation of mutant alleles through the use of chemical mutagens. Erickson et al. (1988) and Fehr et al. (1991a) described the development of the mutant lines C1726 (*fap1*) and A22 (*fap3*), each of which contain an allele for reduced palmitate content of ≈ 70 g kg^{-1}. Fehr et al. (1991b), Schnebly et al. (1994), Stoltzfus et al. (2000b), and Narvel et al. (2000) described the mutant lines A21 (*fap2-b*), A24 (*fap4*), A27 (*fap5*), and A25 (*fap6*), each of which contain an allele for elevated palmitate content of ≈ 160 to 200 g kg^{-1}. Soybean oil with ≈ 250 g kg^{-1} palmitate was developed by combining the *fap2-b* and *fap4* alleles (Fehr et al., 1991b). Oil with elevated palmitate has greater oxidative stability for food and industrial applications than conventional soybean oil that has only ≈ 110 g kg^{-1} palmitate.

A mutant line A30 with ≈ 160 g kg^{-1} palmitate was developed at Iowa State University by treatment of seeds of line A89-144026 with *N*-nitroso-*N*-methyl urea. The inheritance of elevated palmitate in the mutant line has not been reported. The purpose of this research was to describe the inheritance of elevated palmitate in the mutant line A30.

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MATERIALS AND METHODS

A30 was crossed with C1726, A21, A22, A24, A27, and A25 at the Agricultural Engineering and Agronomy Research Center near Ames, IA, during 1996. The soil type at the location is a Nicollet loam (Fine-loamy, mixed, superactive, mesic Aquic Hapludoll). C1726 has the *fap1* allele and A22 the *fap3* allele for reduced palmitate. A21 has *fap2-b*, A24 *fap4*, A27 *fap5*, and A25 *fap6* for elevated palmitate. A reciprocal cross was made between A30 and its parent, A89-144026, to evaluate maternal effects and dominance of the alleles that control palmitate content.

A randomized complete-block design was used to analyze the palmitate content of the F_1 and parent seeds. Each replication consisted of a hybrid seed and a selfed seed from each parent. The seeds were cut into two portions with a razor blade, and the portion of the seed that lacked the embryonic axis was used for fatty ester analysis. Fatty ester content was measured by gas chromatography as described by Hammond (1991).

The portion of the F_1 and parent seeds containing the embryonic axis were planted at the Iowa State University-University of Puerto Rico research nursery at Isabela, PR, in October 1996. The soil type is a Coto clay (Very-fine, koalinitic, isohyperthermic, Typic Haplorthox). Each F_1 and parent plant was harvested individually. A random sample of 110 F_2 seeds from the reciprocal cross and 188 F_2 seeds from each A30 × mutant cross were cut into two portions and analyzed for palmitate content in Ames during January 1997. Five seeds from each of six plants of each parent grown in the same environment also were split and analyzed. The portion of the F_2 and parent seed containing the embryonic axis were planted in Puerto Rico in February 1997. Each F_2 and parent plant was harvested individually. A progeny test of 11 individual F_3 seeds of 50 random F_2 plants and five seeds from each of six plants of each parent were analyzed for palmitate content for the genotypic evaluation of the F_2 plants.

Analysis of the F_2 data indicated that the loci in the A30 × A25 cross may be linked. An additional 1007 F_2 seeds from the cross were cut into two portions and analyzed. The portion of the seeds containing the embryonic axis and seeds of the parents were grown at the Agricultural Engineering and Agronomy Research Center in 1998. An F_2 seed was considered a transgressive segregate if its palmitate content was either less or greater than both parents. Each F_2 and parent plant was harvested individually. Eleven individual F_3 seeds from each F_2 plant identified as a transgressive segregate and five seeds from each of six plants of the two parents were analyzed for palmitate content.

The evaluation of segregation in each cross was based on the mean palmitate content ± two standard deviations of the seed from the parents grown in the same environment. The